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Not for use in diagnostic procedures.

MBL
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MONOCLONAL ANTIBODY

Anti-Podocalyxin (PCLP1) (Human) mAb-PE

Code No.	Clone	Subclass	Quantity
M084-5	53D11	Mouse IgG2a	1 mL (50 tests)

BACKGROUND: Recent studies with avian embryos and murine embryonic stem cells have suggested that hematopoietic cells are derived from hemangioblasts, the common precursors of hematopoietic and endothelial cells. Hara *et al*. molecularly cloned podocalyxin-like protein 1 (PCLP1) as a novel surface marker for endothelial-like cells in the AGM (aorta-gonad-mesonephros) region of mouse embryos, where long-term repopulating hematopoietic stem cells (LTR-HSCs) are known to arise. $PCLP1^{+}CD45^{-}$ cells in the AGM region incorporated acetylated low-density lipoprotein and produced both hematopoietic and endothelial cells when cocultured with OP9 stromal cells. Moreover, multiple lineages of hematopoietic cells were generated in vivo when $PCLP1^{+}$ $CD45^{-}$ cells were injected into neonatal liver of busulfan-treated mice. Today it is reported that the PCLP1 is identical with the Podocalyxin.

SOURCE: This antibody was purified from mouse ascites fluid using protein A agarose. This hybridoma (53D11) was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with CHO cell expressing full-length human Podocalyxin/PCLP1.

FORMULATION: 50 tests in 1 mL volume of PBS containing 1% BSA and 0.09% NaN_3.
*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at 4°C.

REACTIVITY: This antibody reacts with human Podocalyxin/PCLP1 on Flow cytometry.

APPLICATION:
Flow cytometry; 20 µL (ready for use)
*Please refer to the data sheet (MBL, code no. M084-3) for other applications.

Detailed procedure is provided in the following **PROTOCOL**.

INTENDED USE:
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SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	HUVEC	Not tested	Not tested
Reactivity on FCM	+		

REFERENCES:
1) Doyonnas, R., *et al*., *Blood* **105**, 4170-4178 (2005)
2) Minegishi, N., *et al*., *Blood* **102**, 896-905 (2003)
3) Schopperle, M.W., *et al., Biochem. Biophys. Res. Commun.* **300**, 285-290 (2003)
4) Minehata, K., *et al*., *Blood* **99**, 2360-2368 (2002)
5) Doyonnas, R., *et al., J. Exp. Med.* **194**, 13-27 (2001)
6) Hara, T., *et al., Immunity* **11**, 567-578 (1999)
7) Kershaw, B. D., *et al*., *J. Biol. Chem*. **272**, 15708-15714 (1997)

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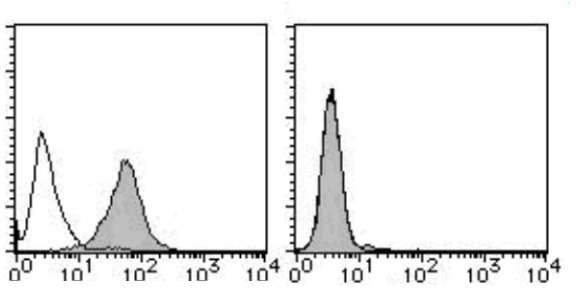

Flow cytometric analysis of PCLP1 expression on HUVEC (left) and CHO (right). Open histogram indicates the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of M084-5 to the cells.

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOL:

Flow cytometric analysis for adherent cells

We usually use Fisher tubes or equivalents as reaction tubes for all steps after 2).
1) Detach the cells from culture dish by using cell dissociation buffer (Invitrogen, code no. 13151-014).
2) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.09% NaN_3].
3) Resuspend the cells with washing buffer (5 x 10^6 cells/mL).

4) Add 50 μL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
5) Add 20 μL of Clear Back (human Fc receptor blocking reagent, MBL, code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
6) Add the primary antibody as suggested in the **APPLICATION**. Mix well and incubate for 30 minutes at room temperature.
7) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
8) Resuspend the cells with 500 μL of washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; HUVEC)